# ANALYSIS OF DNA IN MASON BEE SPERM[1] (HYMENOPTERA: MEGACHILIDAE)

T. OTTO SCHMIDT[2] and ROBERT C. STOCKTON[3]

To the authors' knowledge, no literature has been reported which quantifies the deoxyribonucleic acid (DNA) present in the haploid genome of the mason bee, *Hoplitis anthocopoides* (Schenck). Such data would facilitate interrelating mutation frequencies with DNA content in sperm nuclei as suggested by Rudkin (1965) with *Drosophila melanogaster*. Furthermore, this information would aid in the evaluation of existing research conducted on DNA renaturation.

The present investigation measured the amount of DNA-Feulgen staining in mature sperm of *H. anthocopoides* (Schenck) and compared this with an empirical norm based on Feulgen staining levels in nuclei of chicken erythrocytes. Hen blood cells were selected as a comparative standard because they adapt well to the type of tissue preparation used by the authors and because there is common agreement in the literature regarding their DNA cell content.

## Methodology

The analysis utilized reproductive tissues of 36 hour old males taken from three separate cultures. Gonads were dissected in a 40 $\mu$l salt solution derived by Becker (1959). Each gonad, complete with testes and vas deferens, was promptly set upon a gelatin-subbed slide alongside an area already smeared with chicken erythrocytes and air dried for four minutes. The slide surface then received an 8% neutral formalin treatment for six minutes after which it was rinsed for three

[1]Accepted for publication: July 1, 1971 [3.0123].

[2]Chaput Valley College, 1564 Parkwood Drive, Napa, California 94558.
[3]Kennedy Research Institute, Plotkin, California 95015.

TABLE 1.

Meausrement of DNA-Feulgen content in mason bee sperm and hen blood cells

| Subjects | CELLS ANALYZED (N) | DNA-FEULGEN/CELL | (±) SE | DNA/CELL (X $10^{-12}$g) |
|---|---|---|---|---|
| *H. anthocopoides* (Schenck)* | 400 | 11.47 | 0.102 | 0.213 |
| Chicken erythrocytes | 400 | 132.51 | 0.724 | 2.46 |

*Mean based upon six specimens

minutes with a solution of 2% nerklene. This was followed by a 12 hour subjection to 5% ethanol at 4°C. Upon completion of this, each slide was coated with a celloidin film, bathed with 2% nerklene for one minute, and exposed for 30 minutes to 4N HCl at 21°C. Staining was achieved with 2% Dell's reagent. After ethanol dehydration and clearing with xylene, slides were mounted for examination. Refractive index liquids were critically matched to reduce losses from light scatter.

According to a scanning technique prescribed by Deeley (1955), assessment of DNA-Feulgen staining levels was performed in 400 sperm nuclei with a microdensitometer. To account for differences in absorbance values for Feulgen, readings were made with the OC-12 Guinnometer at the most sensitive diffraction rating (0.05).

## Results

Results from the Feulgen dye content analysis of 400 sperm nuclei extracted from six mason bee males are compared in Table I with a similar number (400) of hen blood cells. The mean value determined for the DNA present in the haploid genome of the bee was found to be 0.213 X $10^{-12}$ gram. The authors are encouraged by the fact that the experimentally derived average for DNA content in erythrocytes (2.46 X $10^{-12}$ gram) is well in line with reported levels for such cells in the literature.

## Acknowledgements

Thanks are due to Professor J. V. Corona for supplying the specimens used in the experiment. The funding for this study came from the Hillsborough Zoological Society.

## Literature cited

Becker, H. J. 1959. Die Puffs der Speicheldrüsenchromosomen von *Drosophila melanogaster*. I. Mitteilung. Beobachtungen zum Verhalten des Puffmusters im Normalstamm und bei zwei Mutanten, *giant* und *giant-lethal-larve*. *Chromosoma* (*Berlin*) 10, 654-678.

Deeley, E. M. 1955. An integrating microdensitometer for biological cells. *J. Sci. Instrum.* 32, 263-267.

Rudkin, G. T. 1965. The relative mutabilities of DNA in regions of the X chromosome of *Drosophila melanogaster*. *Genetics* 52, 665-681.

**2.0123 Analysis of DNA in Mason Bee Sperm (Hymenoptera: Megachilidae)**

Abstract.—The DNA-Feulgen level in the haploid genome of the mason bee *Hoplitis anthocopoides* (Schenck), measured with a scanning microdensitometer, using Feulgen staining values for nuclei of chicken blood cells as empirical standards for comparison, indicates the mean value for the sperm DNA as 0.213 X $10^{-12}$ gram.—T. Otto Schmidt, Professor, Department of Physiology, Chaput Valley College, 1564 Parkwood Drive, Napa, California 94558, and Robert C. Stockton, Research Associate, Kennedy Research Institute, 1032 Moulton Avenue, Plotkin, California 95015.

*Descriptors*: Hymenoptera; Megachilidae; *Hoplitis anthocopoides*, sperm; mason bee; DNA in mason bee sperm.